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Jpn. J. Ent., **62** (4): 709–718. December 25, 1994

A New Cecidomyiid Successor (Diptera) Inhabiting Empty Midge Galls[1)]

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Abstract A new species of the genus *Lasioptera* is described together with information about its bionomics. This gall midge is distinguishable from its relatives by differences in the larval and pupal papillae and by the unique habit as a successor. The larvae inhabit empty galls of other gall-making cecidomyiids, feeding on mycelia of a fungus growing in the galls, and drop to the soil for pupation.

Key words: Cecidomyiidae; *Lasioptera yadokariae*; successor; new species; empty gall.

Introduction

Successors inhabit galls after the gall-making organisms and their associates (parasitoids, predators, and inquilines) have departed (MANI, 1964, as "successori"). As the galls usually do not decay some time after the escape of these organisms, the dry galls provide shelters to a variety of arthropods and breeding ground for fungi. Some of the successors utilize galls as occasional shelters or nests, but others are associated with particular sorts of galls and have become wholly specialized for living in old galls. Many species of arthropods, such as ants, aphids, thrips, and mites, are known to inhabit empty galls (MANI, 1964; PATTON, 1897; PING, 1920; WALSH, 1864/1866). As far as we know, however, no gall midge has been recorded as a successor, although *Lasioptera querciflorae* FELT could be one because it was reared from empty leaf galls of a cynipid on oak in North America (GAGNÉ, 1989).

In an earlier survey of the arthropod community centered upon *Pseudasphondylia neolitseae* YUKAWA on *Neolitsea sericea* (Lauraceae), notice was made of a gall midge, *Lasioptera* sp., whose larvae inhabited empty galls of *P. neolitseae* and dropped to the soil for pupation (YUKAWA, 1983). The species was left unnamed owing to the insufficient number of adult specimens obtained at that time. We have since been able to obtain further specimens, as well as information about its bionomics through field surveys and laboratory experiments. In addition, a comparative morphological study has proved that the successor in galls of *P. neolitseae*

1) Supported in part by a Grant-in-Aid for Scientific Research from the Japan Ministry of Education, Science and Culture (No. 02660052).

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is identical with those in leaf galls produced by *Daphnephila machilicola* YUKAWA on *Machilus japonica* (Luaraceae) and by *Masakimyia pustulae* YUKAWA et SUNOSE on *Euonymus japonicus* (Celastraceae). We have compared the successor species with other relatives of the genus *Lasioptera* in Japan, and have reached the conclusion that it is new to science. In this paper the new species is described as *Lasioptera yadokariae*, and information is given on the galls utilized, life history, behavior, associated fungus, and geographical distribution.

Materials and Methods

Adults of the 3 galling cecidomyiids and their parasitoids normally emerge in April and May in southern Kyushu (see MAEDA *et al.*, 1982; TAKASU & YUKAWA, 1984; and YUKAWA & SUNOSE, 1976 for the life histories of the gallers; see MAETÔ, 1991; SUNOSE, 1984; and YUKAWA, 1983 for the parasitoid species). Field surveys and gall collection were, therefore, performed in April and May during the period from 1971 to 1993 mainly in the following 5 field stations in Kagoshima City: Ishiki, Shiroyama, Ryûgamizu, Toso, and Terayama. Whenever we found adults of the successor in the field, we recorded their activity together with its duration in time. In particular, oviposition behavior, including time required for laying one egg was recorded at the Toso Field Station almost every day from late April to early May in 1987.

Galls inhabited by the successor were easily recognized by the presence of white mycelia plugging the exit holes. Some galls were dissected to obtain larval specimens of the successor and learn the number of larvae and developmental stadia in each gall. Actual data on the number of larvae per gall were compared by χ^2-test with those expected from random (*poisson*) distribution pattern.

To rear adults and know the voltinism, a total of 129 mature larvae (3rd instars) were collected from the Ishiki, Shiroyama, and Toso Stations in April and May 1987. They were divided into 10 groups consisting of 1 to 15 individuals, and each group was placed in a 20 cc vial containing moistened sterilized soil. All (10) vials were incubated under the rearing program shown in Table 1 to subject the mature larvae to 4 artificial seasons in a relatively short period of time. The incubation program was roughly determined according to the climatic conditions in Kagoshima City. The incubation was continued from July 1, 1987 to February 8, 1988, repeating 3 times of artificial annual cycle, to examine the possibility of long term diapause which appears in 2-year or 3-year type life cycle.

About one month before the completion of the 1st cycle, the number of emerging adults was counted hourly for 2 weeks from August 26 to September 9, 1987 to learn the peak emergence time in a day. To determine the natural adult life span, 6 males and 12 females were caged with a small moistened cotton ball in a glass cylinder (18×30 cm) covered at one end by muslin sewn to a wooden ring, and kept under the laboratory conditions. The number of surviving midges was counted

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Table 1. *Lasioptera yadokariae* sp. nov.: Incubation program to make the mature larvae experience the 4 seasons in a relatively short period.

	Period of incubation	Length in days	Temp. (°C)	Lightening time (hr.)
1	July 1～July 7	7	28	13.5
2	July 8～July 14	7	20	11.5
3	July 15～July 21	7	9	10.0
4	July 22～July 24	3	7	10.5
5	July 25～July 26	2	1	10.5
6	July 27～July 28	2	8	11.0
7	July 29～Aug. 6	9	16	12.8
8	Aug. 7～Aug. 17	11	18	12.8
9	Aug. 18～Aug. 25	8	23	12.8
10	Aug. 26～Sep. 31	37	23	13.5
	〈Subtotal〉	〈93〉		
11	Oct. 1～Oct. 7	7	26	14.0
12	Oct. 8～Oct. 14	7	28	13.0
13	Oct. 15～Oct. 21	7	20	11.5
14	Oct. 22～Oct. 28	7	9	10.0
15	Oct. 29～Oct. 31	3	7	10.5
16	Nov. 1～Nov. 2	2	1	10.5
17	Nov. 3～Nov. 4	2	8	11.0
18	Nov. 5～Nov. 10	6	23	11.0
19	Nov. 11～Nov. 26	6	23	12.8
	〈Subtotal〉	〈47〉		
20	Nov. 27～Dec. 4	8	26	14.0
21	Dec. 5～Dec. 6	2	9	10.0
22	Dec. 7～Dec. 9	3	7	10.5
23	Dec. 10～Dec. 11	2	1	10.5
24	Dec. 12～June 6	26	8	11.0
25	June 7～Feb. 8	33	23	12.8
	〈Subtotal〉	〈74〉		

hourly.

For microscopic study, larvae, pupae, and adults were mounted on slides in Canada balsam using ethanol and xylene. Drawings were made with the aid of a camera lucida.

The type specimens are kept in the collection of the Laboratory of Entomology, Faculty of Agriculture, Kagoshima University.

Fungus for identification was isolated from the inner wall of the *P. neolitseae* gall and cultivated on water agar at 20°C in the Laboratory of Plant Pathology, Faculty of Agriculture, Kagoshima University.

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Lasioptera yadokariae sp. nov.

Etymology: "Yadokari" in Japanese means successor.
Japanese name: Yadokari-tamabae.
English name: Leaf midge gall successor.

Male. Eye bridge 6 to 7 facets long medially; fronto-clypeal setae relatively dense (Table 2); palpus pale grayish brown, 4 segmented, about 2/3 as long as height of head, with relatively short setae and scales. Antenna: scape and pedicel pale grayish brown; scape with ventral setae and scales densely, dorsal ones sparsely; flagellomeres dark grayish brown; number of flagellomeres varying from 17 to 26; 1st and 2nd flagellomeres fused; each flagellomere basally with a whorl of scales which are about 2/3 as long as length of basal enlargement. Coxa, femur, and tibia pale grayish brown; tarsus grayish brown; claws of all legs bifid; empodium a little shorter than claw. Wing about 2.1 times as long as wide (Table 2); R_5 straight, joining costa half way between wing base to apex. Abdominal segments densely covered with scales except tergites 7 and 8; a few setae present laterally on tergite 7; setae absent from tergite 8. Genitalia grayish brown; hypoproct (subanal plate)

Table 2. *Lasioptera yadokariae* sp. nov.: fronto-clypeal, mesopleural and mesepimeral setal counts, number of flagellomeres, and measurements of palpus (μm), wing (mm), and flagellomeres (μm).

	♂			♀		
	n	mean±s. d.	(range)	n	mean±s. d.	(range)
Fronto-clypeal setae	7	80.4±11.3	(67–99)	3	83.3±17.8	(63–96)
Mesopleural setae	7	18.4± 5.8	(10–27)	6	20.5± 7.0	(15–32)
Mesepimeral setae	8	10.9± 1.6	(8–13)	8	11.4± 2.6	(7–15)
Palpal segment I	8	14.3± 1.9	(12.5–17.5)	4	14.4± 2.4	(12.5–17.5)
II	8	44.4± 5.3	(37.5–50.0)	4	36.3± 6.0	(32.5–45.5)
III	5	48.5± 4.5	(42.5–55.0)	5	51.5±13.8	(37.5–72.5)
IV	3	61.7± 1.4	(60.0–62.5)	2	70.0±21.2	(55.0–85.0)
Wing length	9	1.34±0.16	(1.13–1.58)	7	1.41±0.15	(1.18–1.58)
Wing width	9	0.63±0.08	(0.55–0.78)	7	0.70±0.07	(0.58–0.75)
length/width	9	2.1 ±0.17	(1.84–2.41)	7	2.0 ±0.08	(1.89–2.12)
No. of flagellomeres	12	21.7± 3.0	(17–26)	7	25.7± 4.5	(17–29)
Flagellomere III						
basal enlargement	10	29.3± 1.7	(27.5–32.5)	8	28.4± 1.9	(25.0–30.0)
maximum width	10	26.5± 1.7	(25.0–30.0)	8	31.3± 4.4	(25.0–37.5)
be/w*	10	1.1±0.14	(0.75–1.18)	8	0.92±0.13	(0.73–1.10)
Flagellomere V						
basal enlargement	10	25.8± 2.1	(22.5–30.0)	8	25.9± 3.0	(20.0–30.0)
maximum width	10	26.5± 1.7	(25.0–30.0)	8	30.6± 3.2	(25.0–35.0)
be/w*	10	0.98±0.12	(0.75–1.20)	8	0.86±0.15	(0.73–1.20)

*: length of basal enlargement/maximum width of basal enlargement.

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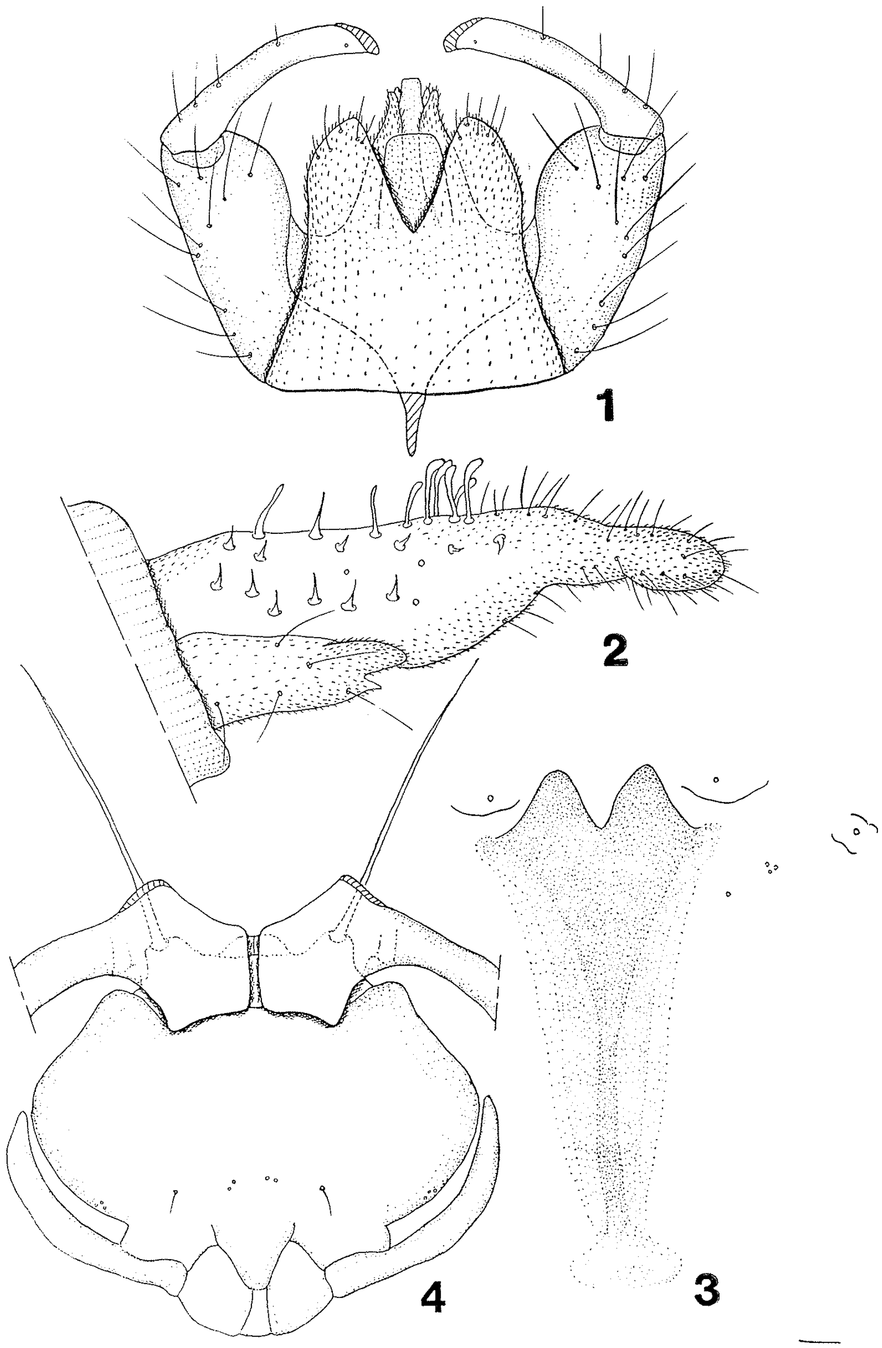
entire, weakly rounded distally, distinctly shorter than aedeagus; gonostylus relatively slender, weakly curved, narrowest at distal 2/3, apically with a strong claw; gonocoxite basally with a setose lobe which is nearly as long as gonocoxite; aedeagus slender, distally truncated (Fig. 1).



Figs. 1–4. *Lasioptera yadokariae* sp. nov. —— 1. Male genitalia; 2. Ovipositor; 3. Sternal spatula and adjacent papillae of mature larva; 4. Basal portion of antennal sheath and frontal area of pupa.

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Female. Wing length about 2.0 times as long as wide (Table 2). Antenna with 17 to 29 flagellomeres. Ovipositor pale brown; a lateral group of many strong, longitudinally flattened setae present immediately posterior to tergite 8; cercus (upper lamella) dorsally with several pairs of fish-hook shaped spines, laterally with short spines which are arranged irregularly in 2 to 3 rows; apical portion of cercus entire, rounded (Fig. 2). Otherwise practically as in male.

Egg. We observed in the field only one egg which was placed on the inner wall of the gall produced by *P. neolitseae*. Subtransparent to pale yellow in color, spheroidal, with the major axis 300 μm and the minor axis 75 μm.

Mature larva: 2nd antennal segment relatively long, about 25 μm in length, about 3.3 times as long as basal width; cervical papillae without setae; 6 dorsal papillae present on each of thoracic and 1st to 7th abdominal segments, all with setae which are 35 to 55 μm; 2 dorsal papillae of 8th abdominal segment with setae which are 50 to 55 μm; pleural papillae with setae about 50 μm long; 8 terminal papillae present, all with setae about 50 μm long; stigmata normal in number and position. Sternal spatula relatively short, 115 to 185 μm, distally bifid (Fig. 3); inner lateral papillae 1, outer lateral papillae 3, all without setae on all thoracic segments; sternal papillae without setae on all thoracic segments; inner pleural papillae without setae on prothorax, with 33 to 38 μm long setae on meso- and metathorax; 4 anterior ventral papillae all without setae; 2 posterior ventral papillae with setae which are 30 to 33 μm; 4 ventral papillae of 8th abdominal segment with setae which are 20 to 23 μm; 4 anal papillae all without setae.

Pupa. Base of antenna slightly developed anteriorly, forming an obtuse angle (Fig. 4); apical papillae with 250 to 330 μm long setae; facial protuberances absent; 2 pairs of posterior facial papillae and a group of 3 lateral facial papillae distinct, an additional pair of facial papillae present between posterior and lateral facial papillae, with relatively long setae (Fig. 4); prothoracic spiracle 225 to 290 μm long; stigmatal tubercles present on 2nd to 6th abdominal segments, each 20 to 30 μm long and 6 to 8 μm wide basally; each abdominal segment with dense minute spinules dorsally, laterally, and ventrally; all abdominal segments except 1st and terminal ones each with several transverse rows of dorsal spines.

Remarks. Fourteen other species of the genus *Lasioptera* are known from Japan (OHNO & YUKAWA, 1984; YUKAWA, 1971), and all of them are gallers on various plant species. The new species is unique in its habit of successor. In addition, it is distinguishable from them by the combination of the following morphological features: (1) ratio of wing length/width is 2.1 ± 0.17 in the males and 2.0 ± 0.08 in the females (Table 2), which are distinctly smaller than that in the other members; (2) ovipositor with 8–9 pairs of fish-hook shaped spines (Fig. 2); (3) setae of every larval papillae are markedly longer than those in other species; (4) on the pupal face, there can be seen a pair of special papillae with relatively long setae, in addition to the normal arrangement of posterior and lateral facial papillae (Fig. 4).

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Specimens examined. Holotype, ♂ (on slide, Cecid. No. E0101); from a successor larva inhabiting empty *Pseudasphondylia neolitseae* gall collected by J. YUKAWA from Ishiki, Kagoshima City, April 30, 1979, reared under laboratory conditions, emerged May 14, 1979. Paratypes, 16 ♂♂, 19 ♀♀, 6 mature larvae, and 12 puparia (on slides, Cecid. Nos. E0102–09, E0111–16, E0120–38, E0201–04, E0211–21, E1801–05); see Table 3 for collection data.

Galls utilized. The following leaf galls were utilized by *L. yadokariae*: *P. neolitseae* galls on *N. sericea*; *D. machilicola* galls on *M. japonica*; and *M. pustulae* galls on *E. japonicus*. These gall-forming midges are normally univoltine, and adults emerge in April and May (MAEDA *et al.*, 1982; YUKAWA, 1974; YUKAWA, 1983; YUKAWA & SUNOSE, 1976).

The gall of *P. neoliteseae* is characterized as follows: brownish swelling on the current leaf; epiphyllous portion apically rounded subconical; hypophyllous portion hemispherical; the larval cavity situated in the center. With its apical spines the pupa pushes open the operculum on the under surface of the gall and crawls half way out of the gall (YUKAWA, 1974).

The leaf gall of *D. machilicola* on *M. japonica* is angular, pear-shaped or club-shaped and is attached to the under surface of the leaf with a thin pedicel. An elongate cylindrical larval cavity is situated in the center and is plugged with a subglobular lid, which falls off when the adult or parasitoid emerges (YUKAWA,

Table 3. *Lasioptera yadokariae* sp. nov.: List of slide-mounted specimens examined.

♂ ♀ L P	Galler and Locality	Col. date of larvae	Leg.	Date of emergence	Cecid No.
0 0 6 0	*Pn*: Shiroyama, Kagoshima City	May 2, 1971	JY	—	E0111–16
5 4 0 0	*Pn*: Ishiki, Kagoshima City	Apr. 30, 1979	JY	May 14, 1979	E0101–09
0 4 0 0	*Dm*: Ishiki, Kagoshima City	May 3–9, 1979	JY	June 1–4, 1979	E0201–04
2 3 0 0	*Mp*: Ryûgamizu, Kagoshima City	Apr. 22–28, 1980	TS	May 15–30, 1980	E1801–05
0 1 0 0	*Pn*: Toso, Kagoshima City	Apr. 23–29, 1987	SH	Aug. 28, 1987	E0120
1 0 0 0	*Pn*: Shiroyama, Kagoshima City	Apr. 29, 1987	SH	Aug. 21, 1987	E0121
4 5 0 8	*Pn*: Ishiki, Kagoshima City	May 5, 1987	JY SH	Aug. 30– Sep. 9, 1987	E0122–38
5 2 0 4	*Dm*: Ishiki, Kagoshima City	May 5, 1987	JY SH	Aug. 24– Sep. 1, 1987	E0211–21

Explanation of abbreviations

L: Mature larva, P: Puparium, *Pn*: *Pseudasphondylia neolitseae*, *Dm*: *Daphnephila machilicola*, *Mp*: *Masakimyia pustulae*, JY: Junichi YUKAWA, TS: Tsukasa SUNOSE, SH: Shigekazu HAITSUKA.

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1974). *D. machilicola* also produces barrel-shaped galls on the under surface of the leaves of a related host plant, *Machilus thunbergii* (Lauraceae), but *L. yadokariae* has never been noticed on this plant, possibly because the larval cavity and the exit hole are too large for the successors to inhabit.

The gall of *M. pustulae* is a suboval blister appearing on the under surface of the leaf. There is frequently seen a slight elevation on the upper surface (YUKAWA & SUNOSE, 1976). The galls are divided into thick and thin types (SUNOSE, 1985). The successor was found in the thick type at the Ryûgamizu Field Station.

Fungus associated with larvae. Without exception, galls inhabited by larvae of *L. yadokariae* contain fungal mycelium on which the larvae feed. During the 1st larval stadium, mycelium of the host fungus is not distinct in the gall, but when the larvae become 2nd instars, the exit hole of the gall is plugged with a thick growth of the mycelium. After mature larvae leave the galls to drop to the ground, the mycelium is weakened and disappears. The fungus was identified as *Pestalotia* sp. (Fungi Imperfecti: Melanconiales: Melanconiaceae).

Emergence, sex ratio, and life span. In southern Kyushu the emergence of adults from the ground usually begins in early April and continues until early May. Laboratory experiments indicated that the emergence of both males and females occurred mostly in the morning between 5:00 and 12:00. A few adults emerged in the afternoon. Sex ratio (% of females) at the time of emergence was about 50. The average life span of unmated males and females was 6.67 ± 2.16 hrs. ($n=6$) and 6.67 ± 2.74 hrs. ($n=12$), respectively.

Oviposition. After mating, the females begin preoviposition flight near the host leaves around 10:00 a.m. It lasts for about an hour under favorable weather conditions. They then start to fly actively under the host leaves in search of empty galls and lay their eggs inside the empty galls one by one. The average time required for one egg-laying was 14.2 ± 6.9 sec. ($n=21$). Usually the pupal skin of the gall-making species is left on the exit hole, but no sign is left by parasitoid emergences. The females of *L. yadokariae* did not show any preference between the holes with or without a puparium. In addition, the females did not distinguish unused empty galls from those already utilized by themselves or other conspecific females. We observed sometimes in the field that a female came back to the gall where she had oviposited several minutes before. There was a case where 2 females oviposited in a gall at the same time.

Life history. The duration of the egg stage is not precisely known. A relative abundance of the 1st instars was 84% on April 11, 1987 (Table 4). The dissection of galls indicated that 2 or 3 individuals of the 1st instars inhabited a gall more frequently than what expected from random distribution (Table 5). The 1st instars rapidly developed into 2nd, and then 48% of the larvae became 3rd instars on May 7, 1987 (Table 4). At this time, there was no case where 2 or more 3rd instars coexist in a gall (Table 5). This may suggest that there was an intraspecific competition among the 1st or 2nd instars. There was no sign of attack by any par-

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Table 4. *Lasioptera yadokariae* sp. nov.: Changes in the stadium structure of the larvae (Data from Kagoshima City in 1987).

Larval stadium	Apr. 11 No. (%)	Apr. 29 No. (%)	May 2 No. (%)	May 7 No. (%)	May 12 No. (%)
1st instar	19 (84)	26 (65)	14 (50)	6 (12)	0 (0)
2nd instar	2 (8)	11 (28)	3 (11)	10 (20)	0 (0)
3rd instar	2 (8)	3 (7)	11 (39)	24 (48)	0 (0)
Escaped from the galls	0 (0)	0 (0)	0 (0)	10 (20)	49 (100)
Total	23 (100)	40 (100)	28 (100)	50 (100)	49 (100)

Table 5. *Lasioptera yadokariae* sp. nov.: number of larvae per gall.

No. of larvae per gall	0	1	2	3	4	Mean
Frequency for 1st instars*	60	7	4	5	0	0.39
Frequency for 3rd instars**	20	10	0	0	0	0.33

* 76 galls were dissected on April 23, 1987; $\chi^2=48.4>12.8$ ($P=0.005$).

** 30 galls were dissected on May 7, 1987; $\chi^2=2.58<7.81$ ($P=0.05$).

asitoid species. All 3rd instars left the galls to drop to the ground by May 12 (Table 4). After mid May, neither successor larvae nor fungus mycelia were found in the empty galls at the field stations. The larvae possibly spin cocoons on the ground and pass through the summer, autumn, and winter, and pupate in the following spring. In addition, 33 out of the 129 larvae collected in April 1987 became adults after being exposed for one cycle of the 4 artificial seasons in the incubator (Table 1). These results suggest that the successor is univoltine, though some larvae collected in April and May 1979 became adults within a month when they were kept in 20 cc vials on a desk in the laboratory. As this happened under an unusually long lighting condition, we cannot conclude that the successor is multivoltine.

It should be noted here that 2 of the 129 larvae became adults after being exposed for 2 cycles of the 4 artificial seasons. This result indicates the possibility that some individuals of *L. yadokariae* have a 2-year life cycle as has been noted for other gall midge species such as *Sitodiplosis mosellana* GÉHIN and *Contarinia tritici* KIRBY some of whose larvae diapause in the ground for more than one year (BARNES, 1958; TSUTSUI, 1956). No adult successor emerged after 3 cycles of the 4 artificial seasons, which means that the remainder of the 129 larvae died during the incubation.

Distribution. The successor was found in the following localities in Japan: Kagoshima (see Table 3); Mt. Tachibana, Fukuoka Prefecture, 12 larvae were collected by Mr. K. MIYAMOTO on May 5, 1978 from the galls produced by *P. neolitseae*; Urawa City, Saitama Prefecture, several adults were obtained by Mr. S. USUBA on April 10–30, 1980 by rearing the successor larvae collected from the galls produced by *P. neolitseae*.

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Acknowledgements

We wish to express our cordial thanks to Dr. R. J. GAGNÉ (Systematic Entomology Laboratory, PSI, Agricultural Research Service, USDA) for his critical reading of the draft, and to Prof. K. ARAI (Laboratory of Plant Pathology, Faculty of Agriculture, Kagoshima University) for identifying the fungus. Useful collection data were given by Mr. K. MIYAMOTO, Dr. T. SUNOSE, and Mr. S. USUBA, to whom we are deeply indebted. Our thanks are also due to Messrs. S. ANRAKU, K. FUJIMOTO, and M. HIGASHI for their help in the field surveys.

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(Received July 18, 1994; Accepted July 30, 1994)

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